❋ BD   OneFlow™ Chronic Lymphoproliferative Diseases Limited Panel

BD OneFlow™ LST—Catalog No. 658619—20 Tests

BD OneFlow™ B-CLPD T1—Catalog No. 659293—20 Tests

CE 2797   **IVD**

1.   INTENDED USE

The BD OneFlow™ tubes for chronic lymphoproliferative diseases (CLPD) are intended for in vitro diagnostic use as a panel of fluorochrome-conjugated antibodies for qualitative flow-cytometric immunophenotyping of mature lymphocyte populations on a BD flow cytometer equipped with:

- A 488-nm blue laser, a 640-nm red laser, and a 405-nm violet laser
- The ability to detect forward scatter (FSC) and side scatter (SSC)
- At least eight-color fluorescence
- Software to acquire and analyze the data

These reagents are used as an aid in the differential diagnosis of hematologically abnormal patients having, or suspected of having, B-cell CLPD, T-cell CLPD, and NK-cell CLPD. These tubes can be used with peripheral blood and bone marrow specimens collected in EDTA or heparin tubes and lymph node specimens. The results should be interpreted by a pathologist or equivalent professional in conjunction with other clinical or laboratory findings.

The BD OneFlow™ CLPD Limited Panel comprises the following BD OneFlow™ tubes:

- BD OneFlow™ LST (Lymphoid Screening Tube)
- BD OneFlow™ B-CLPD T1 (B-cell Chronic Lymphoproliferative Disease Tube 1)

These reagents provide multi-parameter, qualitative results for the antigens listed in Table 1.

Table 1 Antigens targeted by the BD OneFlow™ CLPD Limited Panel tubes

	FITC	**PE**	**PerCP-Cy5.5**	**PE-Cy7**	**APC**	**APC-H7**	**V450**	**V500-C**
LST	CD8 and Igλ	CD56 and Igκ	CD5	CD19 and TCRγ/δ	CD3	CD38	CD20 and CD4	CD45
B-CLPD T1	CD23	CD10	CD79b	CD19	CD200	CD43	CD20	CD45

2.   SUMMARY OF THE TEST

In chronic lymphoproliferative diseases (CLPD), clonogenic events lead to the expansion and accumulation of mature-appearing lymphocytes, which carry a proliferative and/or survival advantage over their normal

counterparts.[1] Thus, the detection of phenotypically abnormal and clonal mature lymphocytes is critical to the diagnosis of CLPD. BD OneFlow™ LST can distinguish hematologically abnormal B cells, T cells, and NK cells from their normal counterparts.

The sample acquisition can be automated using the optional BD FACS™ Universal Loader when used with the BD FACSLyric™ flow cytometer. This assay is not for automated sample preparation. Data analysis can be performed using a pre-defined template and gating, however, reviewing all plots and adjusting gating manually by the user are recommended.

Principle of Operation

The reagents in the BD OneFlow™ CLPD Limited Panel are composed of monoclonal antibodies, each conjugated to a specific fluorochrome. The specimen is added to the reagent tube and incubated, allowing each fluorochrome-conjugated monoclonal antibody in the reagent to bind to a specific antigen on the surface of the cells. After incubation, BD FACS™ Lysing Solution is added to lyse the red blood cells in the sample. Cells are acquired on a BD flow cytometer using the instrument software. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about the cell's size, internal complexity, and relative fluorescence intensity. The instrument software is used to analyze the data and report the result.

3. REAGENT

Reagent Composition

The reagents consist of single-use tubes containing the following fluorochrome-conjugated antibodies in an optimized dried formulation. All of the antibodies have IgG_1 heavy chains and kappa light chains with the exception of CD5, which has IgG_{2a} heavy chains and kappa light chains. The antibody clone names are shown in the following table in the row beneath the antibody.

Table 2 Antibody composition of BD OneFlow™ LST and BD OneFlow™ B-CLPD T1

Reagent	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	V450	V500-C
LST	CD8	CD56	CD5	CD19	CD3	CD38	CD4	CD45
	SK1[2]	MY31[3,4]	L17F12[5,6]	SJ25-C1[7,8]	SK7[6]	HB7[7]	SK3[2]	2D1[9,10]
	Anti-Lambda	Anti-Kappa	–	Anti-TCRγ/δ-1	–	–	CD20	–
	1-155-2[11]	TB28-2[11]	–	11F2[12,13]	–	–	L27[7]	–
B-CLPD T1	CD23	CD10	CD79b	CD19	CD200	CD43	CD20	CD45
	EBVCS-5 [8,14]	HI10a[15]	SN8[16]	SJ25-C1	MRC OX-104[17,18]	1G10[19]	L27	2D1
BD Horizon™ V450, BD Horizon™ V500-C								

The antibodies in BD OneFlow™ LST were chosen for their ability to separate normal lymphocytes into their major subpopulations and to distinguish hematologically abnormal B cells, T cells, and NK cells from their normal counterparts.

The antibodies in BD OneFlow™ B-CLPD T1 were chosen to work in conjunction with the antibodies in BD OneFlow™ LST to distinguish CLL from other B-cell chronic lymphoproliferative diseases in patient specimens.

Refer to the article describing the EuroFlow antibody panels[1] for a full description of the utility of the antibodies chosen for the reagents.

Precautions

- The reagents contain 2-methyl-4-isothiazolin-3-one. These reagents are classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and Regulation (EC) No 1272/2008. Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

	Warning
⚠	H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects.
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. P273: Avoid release to the environment.
Response	P302+P352: IF ON SKIN: Wash with plenty of water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P321: Specific treatment (see Safety Data Sheet). P362+P364: Take off contaminated clothing and wash it before reuse.
Disposal	P501: Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

Storage and Handling

- Store tubes at 2–27 °C in the foil pouch.
- Do not freeze the reagent or expose it to direct light at any time during storage or incubation with cells.
- The dried fluorochrome-conjugated antibodies are stable until the expiration date shown on the pouch and tube labels when stored as directed. Do not use after the expiration date.
- Once the pouch is opened, the dried fluorochrome-conjugated antibodies are stable for 1 month when stored as directed.

   CAUTION Ensure the pouch is completely resealed after removing a tube. The reagent is very sensitive to moisture. Do not remove the desiccant from the reagent pouch.

4.   INSTRUMENTS

The recommended BD systems are outlined in the following table. See the corresponding reagent or instrument user documentation for details.

Table 3 Recommended BD systems

Flow cytometer	Setup beads	Setup software	Analysis software
BD FACSLyric™[a]	BD® CS&T Beads BD® FC Beads 7-Color Kit BD® FC Beads 5-Color Kit BD® FC Beads 2-Color Kit (12-color instrument)	BD FACSuite™ Clinical application v1.4 or later[b]	BD FACSuite™ Clinical application v1.4 or later
BD FACSCanto™ II[c]	BD FACSDiva™ CS&T IVD Beads BD OneFlow™ Setup Beads BD® FC Beads 8-Color Kit for BD OneFlow™ Assays	BD FACSDiva™ software v8.0.1 or later	BD FACSDiva™ software v8.0.1 or later

a. 8-color (4-Blue 2-Red 2-Violet), 10-color (4-Blue 3-Red 3-Violet), or 12-color (4-Blue 3-Red 5-Violet)

b. The Loader can be used with BD FACSuite™ Clinical application v1.5 or later

c. 3-laser, 8-color, 4-2H-2V BD default (4-2H-2V) optical configuration

The BD FACS™ Universal Loader can be used with these products. See the *BD FACSLyric™ System Instructions For Use* for more information. The Loader can be used with BD FACSuite™ Clinical application v1.5 or later.

5. SPECIMEN COLLECTION AND PREPARATION

The reagents can be used for immunophenotyping by flow cytometry of peripheral blood (PB), bone marrow (BM) aspirates, or lymph nodes (LN).

- Collect PB specimens aseptically by venipuncture in BD Vacutainer® K2 or K3 EDTA blood collection tubes, or equivalent, or in lithium or sodium heparin.[20]
- Collect BM specimens in BD Vacutainer® K2 or K3 EDTA blood collection tubes, or equivalent, or in lithium or sodium heparin.
- Collect LN specimens in phosphate buffered saline (PBS), PBS-soaked gauze, or cell culture media, such as RPMI.

  We recommend that you follow guidelines described in consensus protocols for flow cytometric immunophenotyping of hematopoietic malignancies.[21,22]

- PB and BM specimens should be processed up to 24 hours after collection.

  Specimens with large numbers of nonviable cells can give erroneous results due to selective loss of populations and to increased nonspecific binding of antibodies to nonviable cells. Viability of specimens should be assessed. A minimum viability of 75% is recommended.[23]

- Samples should be acquired within 60 minutes of staining if kept at room temperature, protected from light.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[24,25] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

Interfering Conditions

Substances present in the specimen might interfere with the assay:

- Use of therapeutic monoclonal antibodies in patient treatment, such as Rituximab and Alemtuzumab, can interfere with recognition of target antigens by this reagent or deplete the clinically relevant cell populations. This should be considered when analyzing samples from patients treated in this fashion. BD Biosciences has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.

- Avoid using potentially compromised specimens, including clotted, hemolyzed, frozen, or refrigerated specimens.

6. PROCEDURE

Reagents and Materials

Reagents and materials provided

The reagents are provided as single-use tubes in foil pouches. Each kit contains four pouches, each containing five tubes of dried reagent.

Reagents and materials required but not provided

- BD OneFlow™ Assays Installer I (Catalog No. 664225)

  An installer is required to use the BD OneFlow™ reagents with BD FACSuite™ Clinical application. The assays installer includes an acquisition sheet, a laboratory report, a physician report, and a supplemental report used for sample analysis and results reporting. Unless you already have the current BD OneFlow™ Assays Installer, you will have to order the installer the first time you order the reagent.

  The *BD OneFlow™ Application Guide for Chronic Lymphoproliferative Diseases for BD FACSLyric™ Flow Cytometers* is provided with the installer.

- BD OneFlow™ Assay Templates Installer (Catalog No. 659305)

  An installer is required to use the BD OneFlow™ reagents with BD FACSDiva™ software. The templates installer includes two global worksheets: the BD OneFlow™ Acquisition worksheet and the BD OneFlow™ Analysis worksheet. Unless you already have the current BD OneFlow™ Assay Templates Installer, you will have to order the installer the first time you order the reagent. The installer also contains the OneFlow Setup template.

  The *Instrument Setup Guide for BD OneFlow™ Assays*, the *BD OneFlow™ LST Application Guide*, and the *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases* are provided with the installer.

- BD FACS™ Lysing Solution (10X) (Catalog No. 349202)

  See the *BD FACS™ Lysing Solution* instructions for use (IFU) for precautions and warnings.

- 15-mL or 50-mL conical polypropylene tubes
- 40-µm cell strainer (for processing BM and LN specimens)
- 1 cc or 3 cc syringe (for processing LN specimens)
- Pasteur pipette
- Serological pipette
- Micropipettor with tips
- Vortex mixer
- Centrifuge
- Wash buffer (filtered PBS + 0.5% BSA + 0.09% or 0.1% sodium azide)
- (Optional) BD FACS™ Universal Loader

NOTE Labs must validate any deviations from the following procedures.

Installing the Assay or Template

The BD OneFlow™ Assay Installer, used with BD FACSuite™ Clinical application, or the BD OneFlow™ Assay Template Installer, used with BD FACSDiva™ software, has to be installed before you run the assay for the first time. Additional assays or templates can be installed at the same time, as needed. If you will analyze the FCS files on a different workstation from the one used to acquire the samples, ensure that you install the assays or templates on both workstations.

To install the BD OneFlow™ assay in BD FACSuite™ Clinical application:

NOTE When you select an assay to install, it will overwrite the BD OneFlow™ assay that was previously installed on the system. If you do not want an existing assay on your computer to be overwritten, do not select that assay from the installer during the installation process.

1. Insert the installer and click the installer icon.

   The InstallShield Wizard for BD OneFlow™ Assays opens.
2. Click **Next**.

   The license agreement opens.
3. Select the **I accept the terms in the license agreement** option and click **Next**.
4. To install all of the assays included on the installer, select the **Complete** option and click **Next**.
5. Optional: To install a subset of the assays included on the installer, select the **Custom** option and click **Next**.

   The **Custom Setup** dialog opens.

   - Click the menu to the left of the appropriate assay.

   - From the menu, select **This assay will be installed on your local hard drive**.
6. Click **Install**.

   The assays will be installed in the Library.
7. Click **Finish**.

   The InstallShield Wizard closes.
8. Optional: Double-click the ReadMe file found on the installer.

   The ReadMe file opens.
9. Click the close box when finished reading it.
10. Remove the installer.

To install the OneFlow template in BD FACSDiva™ software:

NOTE When you select a template to install, it will always overwrite any template with the same name that was previously installed on the system. If you do not want an existing template on your computer to be overwritten, do not select that template from the installer during the installation process.

1. Insert the installer and click the installer icon.
2. Follow the instructions in the dialog.

   The installer will copy and paste the templates in the folder D:\BDExport\Templates\Panel\BD Panels.

   NOTE If your system has only one drive, the templates will be installed in C:\BDExport\Templates\Panel\BD Panels.

   After installation is complete, a dialog opens, summarizing which templates have been successfully copied into the folder.
3. Click **OK** to close the dialog.
4. The installer ReadMe file opens. Click the close box when you have finished reading it.
5. Remove the installer.

Setting up the Cytometer

For BD FACSLyric™ flow cytometers:

1. Use BD® CS&T Beads and BD FACSuite™ Clinical application v1.4 or later, to perform Characterization QC (CQC) every 6 months or as needed, perform daily Performance QC (PQC), and perform daily assay and tube settings setup. For assay and tube settings setup, select the **Run Setup** and **Generate Reports** checkboxes.
2. Use the BD® FC Beads 7-Color Kit, BD® FC Beads 5-Color Kit, and BD FACSuite™ Clinical application v1.4 or later, to update reference settings every 60 days. In addition, use the BD® FC Beads 2-Color Kit to set up a 12-color instrument.

   See the *BD FACSLyric™ System Instructions For Use*, the *BD FACSLyric™ Clinical Reference System*, and the appropriate reagent IFU for more information.

For BD FACSCanto™ II flow cytometers:

1. Use BD FACSDiva™ CS&T IVD Beads (CS&T IVD beads) and BD FACSDiva™ software v8.0.1 or later, to define the baseline of the cytometer and to run a daily performance check of the cytometer.
2. Use BD OneFlow™ Setup Beads, lysed washed blood, andBD FACSDiva™ software v8.0.1 or later, to set photomultiplier tube (PMT) and scatter voltages monthly.
3. Use BD® FC Beads and BD FACSDiva™ software v8.0.1 or later, to set fluorescence compensation monthly.
4. We recommend that you confirm that the PMT voltages (PMTVs) are still within their daily target ranges.

   See the *Instrument Setup Guide for BD OneFlow™ Assays* and the appropriate reagent IFU for more information.

Diluting BD FACS™ Lysing Solution

Dilute the 10X concentrate 1:10 with room temperature (20–25 °C) deionized water. The prepared solution is stable for 1 month when stored in a glass or high density polyethylene (HDPE) container at room temperature.

Processing the Specimen

Washing the specimen

NOTE Before washing the specimen, confirm that the cytometer has been properly set up.

1. For each tube to be stained, label a 15-mL conical tube with the specimen ID.
2. Prepare each specimen type as described:
   - For PB specimens, invert the collection tube 10 times to mix well.
   - For BM specimens, invert the collection tube 10 times to mix well. Verify that the specimen is not lipemic and does not have particulate matter in it.
     – If needed, pass sufficient specimen through a 40-μm cell strainer.
   - For LN specimens, if the specimen is not a single-cell suspension,
     – Place LN in a 40-μm cell strainer set on a 15- or 50-mL conical tube.
     – Gently disrupt the LN to obtain a single-cell suspension by pressing with the plunger of a 1 cc or 3 cc syringe.
     – Wash the cell strainer with wash buffer. Discard the cell strainer.
     – If needed, centrifuge at 540*g* for 5 minutes at 20–25 °C. Remove the supernatant.
     – Resuspend the cell pellet in an appropriate volume of wash buffer.
   - Fine-needle aspirates (FNA) are ready for staining. There is no need to wash the specimen.
3. Add 300 μL of the specimen to each labeled conical tube.
4. Add 10 mL of wash buffer (filtered PBS + 0.5% BSA + 0.09% or 0.1% sodium azide).

5. Invert the tube 3–5 times to mix well.
6. Centrifuge at 540g for 5 minutes at 20–25 °C.
7. Remove the supernatant without disturbing the cell pellet.

    NOTE If the cell pellet is disturbed, centrifuge the tube again and then remove the supernatant without disturbing the cell pellet.
8. Vortex the tube until no cell aggregates remain in the residual wash buffer.
9. Repeat steps 7–11 twice for a total of three washes.
10. Resuspend the cell pellet in wash buffer to give a final volume of 300 µL.
11. Vortex vigorously 3–5 seconds to completely resuspend the cell pellet.

    NOTE Start staining the specimen with the BD OneFlow™ reagent within 30 minutes of the last wash. Store the washed specimen at 20–25 °C until you stain it.

Staining the specimen

1. If the pouch is stored refrigerated, allow it to reach room temperature before opening it.

    NOTE The reagent is very sensitive to moisture. To avoid condensation, open the pouch only if it is at room temperature.
2. For each patient specimen, remove a reagent tube from the pouch.
3. Place the tubes in a rack, protected from light.

    Start staining the specimen within 1 hour of removing a tube from the pouch.
4. Immediately reseal the pouch with any unused tubes.

    NOTE Ensure the pouch is completely resealed after removing a tube. The reagent is very sensitive to moisture. Do not remove the desiccant from the reagent pouch. Use the tubes within 1 month of opening the pouch.
5. Write the patient ID on the tube label within the area provided.
6. Vortex washed specimen 3–5 seconds to mix well.
7. Add 100 µL of washed specimen to the tube. Vortex vigorously 3–5 seconds to mix well.

    If less than 100 µL of specimen is used, add wash buffer to a final volume of 100 µL.

    NOTE Staining from 2.5×10^5 to 4×10^7 white blood cells/mL gives equivalent results.
8. Incubate for 30–35 minutes at 20–25 °C, protected from light.
9. Add 2 mL of 1X BD FACS™ Lysing Solution. Vortex 3–5 seconds to mix well.
10. Incubate for 8–12 minutes at 20–25 °C, protected from light.
11. Centrifuge at 540g for 5 minutes at 20–25 °C.
12. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
13. Vortex vigorously until the cell pellet is completely resuspended.
14. Add 2 mL of wash buffer to the tube. Vortex 3–5 seconds to mix well.
15. Centrifuge at 540g for 5 minutes at 20–25 °C.
16. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
17. Vortex 3–5 seconds to resuspend the cell pellet.
18. Add 200 µL of wash buffer to the tube. Vortex 3–5 seconds to mix well.

    NOTE Samples should be acquired within 60 minutes if kept at room temperature, protected from light.

Setting up the Assay (BD FACSLyric™ Flow Cytometer)

To add a reagent lot ID and expiration date to the library:

1. From the BD FACSuite™ Clinical application navigation bar, click the Library icon.

    The Library workspace opens.
2. Expand the **Beads and Reagents** menu and select **Reagents**.
3. Select the appropriate BD OneFlow™ reagent from the **Product Name** list.

The **OneFlow reagent** pane opens at the bottom of the page.

4. Click **Add Lot**.

   The **Add New Lot** dialog opens.

5. In BD FACSuite™ Clinical application v1.5, click **Scan Barcode** and then scan the barcode on the pouch or tube label.

   The Lot ID and expiration date are entered in the appropriate fields.

   NOTE In BD FACSuite™ Clinical application v1.4, add the Lot ID and expiration date manually.

6. Select the **Current Lot** checkbox.
7. Click **OK**.

   The lot ID and expiration date are added to the appropriate columns for the reagent.

   NOTE Make sure to add the reagent lot and expiration date prior to acquisition. This has to be done only once for a particular reagent lot.

To create a worklist:

1. From the BD FACSuite™ Clinical application navigation bar, click the Worklists icon.

   The Worklists workspace opens.

2. In the **Manage Worklists** tab, click **New**.

   A blank worklist opens in a new tab.

3. In the **Worklist Entries** section, select the appropriate task from the **Task** menu.
4. Enter the **Sample ID** for BD OneFlow™ reagent tasks.

   Do not scan the barcode, found on the tube label, into the software.

   NOTE Multiple lots of the same reagent cannot be run on the same worklist.

5. In the **Loading Options** section, select **Manual** or **Universal Loader** from the **Loading Option** menu.
6. If using the Loader, select **30 Tube Rack** or **40 Tube Rack** from the **Carrier Type** menu.

   NOTE Confirm that the order of samples in the rack matches the order entered in the software when setting up the Loader.

   See the *BD FACSLyric™ System Instructions For Use* for more information.

Setting up the Experiment (BD FACSCanto™ II Flow Cytometer)

1. From the menu bar, select **Edit > User Preferences**, then navigate to the **FCS** tab, and select **Export FCS after recording**, to automatically export the FCS files after acquisition. Click **OK**.
2. Confirm that the cytometer is in the default 4-2H-2V configuration.
3. From the menu bar, select **Experiment > New Experiment > Blank Experiment**. Click **OK**.

   NOTE You can also create an experiment directly from the **Browser** using the **Experiment** icon.

4. If prompted by the **CST Mismatch** window, select **Use CST Settings**.
5. Rename the experiment according to your laboratory practice.
6. In the **Browser**, right-click **Cytometer Settings > Link Setup** and select the appropriate compensation matrix calculated using BD® FC Beads within the past 31 days. Click **Link**.

   See the *BD® FC Beads 8-Color Kit for BD OneFlow™ Assays* IFU or the *Instrument Setup Guide for BD OneFlow™ Assays*.

7. If prompted by the **Cytometer Settings Mismatch** window, select **Overwrite**.
8. Right-click **Cytometer Settings > Unlink From** the previously linked compensation setup. Click **OK**.

   NOTE Unlinking the compensation setup allows updated application settings to be applied while retaining compensation values.

9. In the **Browser**, right-click **Cytometer Settings > Application Settings > Apply** and select the most recent application settings determined within the last 31 days using the BD OneFlow™ Setup Beads. Click **Apply**.

10. A Confirm dialog opens. Select **Keep the compensation value**.
11. If prompted by the **Confirm Cytometer Changes** dialog, click **Yes** to overwrite the cytometer values for **FSC Area Scaling**.
12. From the menu bar, select **Experiment > New Specimen**.

    The **Panel Templates** dialog opens.
13. Navigate to the **BD Panels** tab and select the OneFlow LST template.
14. Indicate the number of patient specimens you want to acquire using the **Copies** field near the bottom of the **BD Panels** tab. Click **OK**.
15. Rename each specimen, for example, with the appropriate patient ID in front of the specimen name.

    NOTE If you have to re-run a particular patient sample, set the current tube pointer to the tube you wish to re-run. Click **Next Tube** in the **Acquisition Dashboard** to create another tube for that patient. Do not select **Experiment > New Tube** from the menu bar or use the **New Tube** icon from the **Browser** menu bar to create the additional tube to be acquired because the labels and barcode fields will not be populated.

    NOTE If you want to acquire additional patient samples stained with the BD OneFlow™ reagent in the experiment, repeat steps 12–15 to add new specimens.

    Two **Confirm** dialogs will open asking if you want to create another acquisition worksheet or another analysis worksheet. Click **Cancel** in each dialog.
16. From the menu bar, select **Experiment > Experiment Layout** and navigate to the **Keywords** tab.
17. Highlight the **Product ID** keyword for the appropriate tube, and scan the barcode on the BD OneFlow™ tube label.

    NOTE If you cannot scan the barcode on the tube label, see Troubleshooting.
18. Manually add the appropriate information to the remaining keywords, as needed.
19. Click **OK** to close the **Experiment Layout**.

Acquiring the Stained Sample

For BD FACSLyric™ flow cytometers:

Two versions of the assay are available:

Assay version	Software	Stopping time	Acquisition mode
v1.0	BD FACSuite™ Clinical application v1.4 or later	5 minutes	Manual
v1.1	BD FACSuite™ Clinical application v1.5 or later	3 minutes	Manual, Loader

The assay will automatically collect 100,000 total events. You cannot append the number of events to collect after acquisition has started. Therefore, if needed, change the number of events to collect before you start acquisition. To change the number of events to collect, see the *BD OneFlow™ Application Guide for Chronic Lymphoproliferative Diseases for BD FACSLyric™ Flow Cytometers*. A clinically relevant number of cells can be determined at the discretion of an appropriate healthcare professional.

To acquire the sample using the BD OneFlow™ assay v1.1:

1. In the **Worklist Controls** bar, select **Run All** from the **Run** menu to run the entire worklist from the beginning.

   Alternatively, to acquire a specific tube, set the run pointer to the sample you want to run and select **Run from Pointer** from the **Run** menu.
2. Vortex each stained tube 3–5 seconds at low speed immediately prior to acquisition.

   If using the BD FACS™ Universal Loader, vortex tubes immediately before placing them in the Loader racks.

NOTE Make sure that all of the BD OneFlow™ tubes in the rack are acquired within 1 hour. If not, you must validate tubes acquired outside the 1 hour time.

3. Follow the prompts in the software to load or unload tubes.

   The BD OneFlow™ Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Leukocytes, Lymphocytes, and the relevant cell populations for the assay.

4. Examine each dot plot on the acquisition sheet.

   NOTE The preview time is 10 seconds and then data is automatically recorded. Do not increase the preview time and risk loss of the sample due to insufficient volume.

   NOTE The assay will automatically collect 100,000 total events. If the assay cannot collect 100,000 total events, acquisition will stop after 3 minutes. A QC message, "All Events gate does not contain the requested 100,000 events" is generated in the Lab Report, and can be ignored if the sample can be analyzed using the events acquired.

   See the *BD FACSLyric™ System Instructions For Use* for more information.

To acquire the sample using the BD OneFlow™ assay v1.0:

1. In the **Worklist Controls** bar, select **Run All** from the **Run** menu to run the entire worklist from the beginning.

   Alternatively, to acquire a specific tube, set the run pointer to the sample you want to run and select **Run from Pointer** from the **Run** menu.

2. Vortex each stained tube 3–5 seconds at low speed immediately prior to acquisition.
3. Follow the prompts in the software to load or unload tubes.

   The BD OneFlow™ Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Leukocytes, Lymphocytes, and the relevant cell populations for the assay.

4. Examine each dot plot on the acquisition sheet.

   NOTE The preview time is 10 seconds and then data is automatically recorded. Do not increase the preview time and risk loss of the sample due to insufficient volume.

5. If it appears that fewer than 100,000 events will be collected, monitor the sample volume and click **Stop Tube** in the **Worklist Controls** bar to stop acquisition before the tube runs dry.

   NOTE The assay will automatically collect 100,000 total events. If the assay cannot collect 100,000 total events, acquisition will stop after 5 minutes. However, make sure you monitor the sample volume and click **Stop Tube** in the **Worklist Controls** bar to stop acquisition before the tube runs dry. To change the stopping criteria, see *the BD OneFlow™ Application Guide for Chronic Lymphoproliferative Diseases for BD FACSLyric™ Flow Cytometers.*

   See the *BD FACSLyric™ System Instructions For Use* for more information.

For BD FACSCanto™ II flow cytometers:

1. In the Browser, expand the appropriate specimen and set the current tube pointer to that tube.
2. Select the appropriate **BD OneFlow™ Acquisition** worksheet tab.
3. Vortex the stained tube 3–5 seconds at low speed.
4. Install the tube on the cytometer. Adjust the flow rate to **Medium** in the **Acquisition Dashboard**. Click **Acquire Data**.
5. Verify that the population is on scale and adjust the gate in the first plot of the acquisition worksheet to exclude debris, if needed.
6. Click **Record Data** in the **Acquisition Dashboard** to collect total events.

   NOTE The template will automatically collect 100,000 total events. Use the menu in the **Acquisition Dashboard** to select a different number of events to acquire, if needed. A clinically relevant number of cells can be determined at the discretion of an appropriate healthcare professional.

7. Inspect the dot plots on the acquisition worksheet and adjust the gates as needed.

Some of the dot plots might look different from those in other experiments. The initial FSC-A vs SSC-A dot plot to identify cells and eliminate debris may appear compressed. This is a consequence of the target values used to create the application settings. The values are specified by the EuroFlow Consortium.

NOTE Enlarge the dot plots while adjusting the gates so you can more readily see the populations of interest. After adjusting the gates, collapse the dot plot to its original size.

The FSC-A vs SSC-A dot plot is used to identify cells.

The remaining dot plots either contain gates to identify the relevant cell populations for the assay, or do not contain gates and are included to ensure that the antibodies can stain cells in the specimen, therefore serving as an internal quality control for the tube.

See the appropriate BD OneFlow™ Application Guide for examples of the dot plots showing populations of normal cells in the acquisition worksheet.

8. Acquire the next sample.
9. From the menu bar, select **File > Export > Experiments**, and select the **Directory Export** option. Click **OK**.

Analyzing the Data Using BD FACSuite™ Clinical Application

NOTE FCS files acquired using BD OneFlow™ assay v1.0 can be opened in BD OneFlow™ assay v1.1.

To analyze a sample stained using BD OneFlow™ LST:

1. Set the run pointer to the appropriate sample in the **Worklist Entries** panel.

   The BD OneFlow™ LST Laboratory Report opens in the **Laboratory Report** tab.
2. Review the BD OneFlow™ LST Laboratory Report.

   The lab report comprises six pages.

   – The first page of the report shows sample and tube information, population statistics, and QC messages, if generated.

   Populations with a low number of events might report %Parent or %Grandparent as 0.0%. This is due to rounding the result to a single decimal place in BD FACSuite™ Clinical application.

   – The dot plots on the second page of the lab report are used to exclude debris and doublets and to identify the major cell populations: leukocytes, lymphocytes, B cells, T cells, TCRγδ$^+$ T cells, and TCRγδ$^-$ T cells.

   The gates in the dot plots of the BD OneFlow™ LST Laboratory Report are provided for analyzing normal and abnormal cell populations in the specimen.

   Enlarge the dot plots while adjusting the gates so you can more readily see the populations of interest. After adjusting the gates, collapse the dot plot to its original size. See the *BD OneFlow™ Application Guide for Chronic Lymphoproliferative Diseases for BD FACSLyric™ Flow Cytometers* for examples of dot plots showing populations of normal cells.

   – The dot plots on the third page of the lab report are used to identify the Igκ$^+$ and Igλ$^+$ B cells and to further characterize the B-cell population.

   – The dot plots on the fourth page of the lab report are used to divide the TCRγδ$^-$ T cells into CD4$^+$CD8$^-$, CD8$^+$CD4$^-$, CD4$^+$CD8$^+$, and CD4$^-$CD8$^-$ populations. Dot plots are also used to further characterize the TCRγδ$^+$ and TCRγδ$^-$ T cell populations.

   – The dot plots on the fifth page of the lab report are used to identify and characterize NK cells.

   – The last page of the laboratory report includes lot and expiration dates for BD$^®$ CS&T Beads and the BD OneFlow™ reagent, Characterization QC, reference settings, tube settings, and cytometer configuration.

3. (Optional) Select the **Physician Report** tab to view the report.

The OneFlow Physician Report contains a high level summary of the assay results.

4. (Optional) Select the **Supplemental Report** tab to add additional dot plots to further analyze the sample.

   See the *BD OneFlow™ Application Guide for Chronic Lymphoproliferative Diseases for BD FACSLyric™ Flow Cytometers* for more information.

Any gated regions deleted in this Supplemental Report are reflected in the Laboratory and Physician Reports. Any gated regions created in this Supplemental Report might be reflected in the Laboratory Report.

Do not add dot plots or gates to the Laboratory Report or Physician Report. They cannot be deleted and will invalidate the report.

5. Select the **Laboratory Report** tab.
6. Click **E-Sign**.

   The E-Signature dialog opens.

7. Select a user ID.
8. Type your password.
9. (Optional) Enter any comments.
10. Click **Sign**.

    The signer's user ID, date and time, and comments are added to the E-signature box in all three reports.

    See the *BD FACSLyric™ System Instructions For Use* for more information and export options.

To analyze a sample stained using BD OneFlow™ B-CLPD T1:

1. Set the run pointer to the appropriate sample in the **Worklist Entries** panel.

   The BD OneFlow™ B-CLPD T1 Laboratory Report opens in the **Laboratory Report** tab.

2. Review the BD OneFlow™ B-CLPD T1 Laboratory Report.

   The lab report comprises four pages.

   – The first page of the report shows sample and tube information, population statistics, and QC messages, if generated.

     Populations with a low number of events might report %Parent or %Grandparent as 0.0%. This is due to rounding the result to a single decimal place in BD FACSuite™ Clinical application.

   – The dot plots on the second page of the lab report are used to exclude debris and doublets and to identify leukocytes and lymphocytes.

     Enlarge the dot plots while adjusting the gates so you can more readily see the populations of interest. After adjusting the gates, collapse the dot plot to its original size. See the *BD OneFlow™ Application Guide for Chronic Lymphoproliferative Diseases for BD FACSLyric™ Flow Cytometers* for examples of dot plots showing populations of normal cells.

   – The dot plots on the third page of the lab report are used to identify and characterize B cells.

   – The last page of the lab report shows lot and expiration dates for BD® CS&T Beads and the BD OneFlow™ reagent, Characterization QC, reference settings, tube settings, and cytometer configuration.

3. (Optional) Select the **Physician Report** tab to view the report.

   The BD OneFlow™ B-CLPD T1 Physician Report contains a high level summary of the assay results.

4. (Optional) Select the **Supplemental Report** tab to add additional dot plots to further analyze the sample.

   See the *BD OneFlow™ Application Guide for Chronic Lymphoproliferative Diseases for BD FACSLyric™ Flow Cytometers* for more information.

Any gated regions deleted in this Supplemental Report are reflected in the Laboratory and Physician Reports. Any gated regions created in this Supplemental Report might be reflected in the Laboratory Report.

Do not add dot plots or gates to the Laboratory Report or Physician Report. They cannot be deleted and will invalidate the report.

5. Select the **Laboratory Report** tab.
6. Click **E-Sign**.

   The E-Signature dialog opens.

7. Select a user ID.
8. Type your password.
9. (Optional) Enter any comments.
10. Click **Sign**.

   The signer's user ID, date and time, and comments are added to the E-signature box in all three reports.

   See the *BD FACSLyric™ System Instructions For Use* for more information and export options.

Analyzing the Data Using BD FACSDiva™ Software

To analyze a sample stained using BD OneFlow™ LST:

1. From the menu bar, select **File > Import > Experiments**.
2. Select the experiment that you want to analyze. Click **Import**.

   The experiment with the associated acquisition and analysis worksheets opens.

3. Select the **BD OneFlow™ LST Analysis** worksheet tab.
4. Inspect the dot plots on page 1 of the LST analysis worksheet and adjust the gates as needed.

   Some of the dot plots might look different from those in other experiments. The initial FSC-A vs SSC-A dot plot to identify cells and eliminate debris may appear compressed. This is a consequence of the target values used to create the application settings. The values are specified by the EuroFlow Consortium.

   NOTE Enlarge the dot plots while adjusting the gates so you can more readily see the populations of interest. After adjusting the gates, collapse the dot plot to its original size.

   The first three dot plots on the LST analysis worksheet identify cells, FSC singlets, and SSC singlets. Debris and doublets are excluded by adjusting the gates.

   Examine the leukocyte and lymphocyte populations in the CD45 V500-A vs SSC-A dot plot.

   Examine the B-cell and T-cell populations in the CD3 APC-A vs CD19+TCRgd PECy7-A dot plot from the lymphocyte population. Examine the $TCR\gamma/\delta^+$ and $TCR\gamma/\delta^-$ populations in the CD3 APC-A vs CD19+TCRgd PE-Cy7-A dot plot from the T-cell population. The CD38 APC-H7-A vs SSC-A dot plot is included for informational purposes to allow for the visualization of $CD38^+$ cells.

   NOTE See the *BD OneFlow™ LST Application Guide* for examples of dot plots showing populations of normal cells.

5. Inspect the dot plots on page 2 of the LST analysis worksheet and adjust the gates as needed.

   The dot plots on page 2 of the analysis worksheet identify various populations of T cells. $TCR\gamma/\delta^-$ T cells are divided into

   $CD8^+CD4^-$, $CD4^+CD8^+$, $CD4^+CD8^-$, and $CD4^-CD8^-$ populations in the

   CD20+CD4 V450-A vs CD8+IgL FITC-A dot plot.

   The remaining dot plots further characterize $TCR\gamma/\delta^-$ and $TCR\gamma/\delta^+$ cells using various markers.

6. Inspect the dot plots on page 3 of the LST analysis worksheet and adjust the gates as needed.

   The dot plots on page 3 of the analysis worksheet identify B cells. B cells are initially identified as being $CD3^-CD19^+CD45^+$. Examine the level of CD20 expression in the CD19+TCRgd PE-Cy7-A vs CD20+CD4 V450-A dot plot.

   Examine the ratio of Igκ- to Igλ-expressing B cells in the CD56+IgK PE-A vs CD8+IgL FITC-A dot plot.

   The remaining dot plots further characterize B cells using various markers.

7. Inspect the dot plots on page 4 of the LST analysis worksheet and adjust the gates as needed.

   The dot plots on page 4 of the analysis worksheet identify NK cells. NK cells are identified from the NOT(T cells OR B cells) population in the CD45 V500-A vs CD56+IgK PE-A dot plot.

   The remaining dot plots further characterize NK cells using various markers.

8. Examine the results in the statistics box on page 5 of the LST analysis worksheet.

   Confirm that all of the keywords are present in the statistics box. If any of the keywords are missing, see [Troubleshooting](#).

9. Perform further analyses as needed.

   NOTE The gates in the dot plots of the LST analysis worksheet are provided for analyzing normal and aberrant cell populations in the specimen.

10. Save the LST analysis worksheet as a PDF.

    NOTE The analysis worksheet is a global worksheet. Any gates that are adjusted when analyzing a sample on a global worksheet will be changed in previously analyzed files. Previously saved PDFs won't change, but if you go back to a previously analyzed global worksheet, you will have to readjust the gates so they match what they were before.

11. (Optional) Click **Print** to print the LST analysis worksheet.

    Analyze the next sample.

BD OneFlow™ B-CLPD T1

1. Select the **BD OneFlow™ B-CLPD T1 Analysis** worksheet tab.
2. Inspect the dot plots on page 1 of the analysis worksheet, and adjust the gates as needed.

   Some of the dot plots might look different from those in other experiments. The initial FSC-A vs SSC-A dot plot to identify cells and eliminate debris may appear compressed. This is a consequence of the target values used to create the application settings. The values are specified by the EuroFlow Consortium.

   NOTE Enlarge the dot plots while adjusting the gates so you can more readily see the populations of interest. After adjusting the gates, collapse the dot plot to its original size.

   The first three dot plots on the analysis worksheet identify cells, FSC singlets, and SSC singlets. Debris and doublets are excluded by adjusting the gates.

   Examine the leukocyte and lymphocyte populations in the CD45 V500-A vs SSC-A dot plot.

   NOTE See the *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases* for examples of dot plots showing populations of normal cells in the analysis worksheet.

3. Examine the B-cell population in the CD19 PE-Cy7-A vs CD20 V450-A dot plot on page 2 of the analysis worksheet and adjust the gate as needed.

   The B cells are further characterized according to the levels of CD23, CD10, CD79b, CD200, CD43, and CD45 expression in the remaining dot plots.

4. Examine the results in the statistics box on page 3 of the analysis worksheet.

   Confirm that all of the keywords are present in the statistics box. If any of the keywords are missing, see [Troubleshooting](#).

5. Perform further analyses as needed.

   NOTE The gates in the dot plots of the analysis worksheet are provided for analyzing normal and aberrant cell populations in the specimen.

   NOTE When evaluating a patient sample, you must analyze FCS files acquired from the patient sample stained with BD OneFlow™ LST as well as with BD OneFlow™ B-CLPD T1. The markers present in BD OneFlow™ B-CLPD T1 are used in conjunction with CD5, CD38, Anti-Kappa, and Anti-Lambda, present in BD OneFlow™ LST, to differentially diagnose CLL versus other B-cell chronic lymphoproliferative diseases.

See the *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases* for examples of dot plots showing populations of normal cells in the analysis worksheet.

6. Save the BD OneFlow™ B-CLPD T1 analysis worksheet as a PDF.

   NOTE The BD OneFlow™ B-CLPD T1 analysis worksheet is a global worksheet. Any gates that are adjusted when analyzing a sample on a global worksheet will changed in previously analyzed files. Previously saved PDFs won't change, but if you go back to a previously analyzed global worksheet, you will have to readjust the gates so they match what they were before.

7. (Optional) Click Print to print the analysis worksheet.
8. Analyze the next sample.

7. RESULTS

Representative Data

See the appropriate application guide to see laboratory reports showing dot plots from a hematologically normal adult sample stained with each reagent.

For BD FACSLyric™ flow cytometers:

- *BD OneFlow™ Application Guide for Chronic Lymphoproliferative Diseases for BD FACSLyric™ Flow Cytometers*

For BD FACSCanto™ II flow cytometers:

- *BD OneFlow™ LST Application Guide*
- *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases*

8. LIMITATIONS

- Use of this reagent for diagnostic evaluation of hematologic disorders should be performed in the context of a thorough immunophenotypic analysis including other relevant markers.
- Use of the reagents requires experience with leukemia and lymphoma immunophenotyping and classification. The results should be interpreted by a pathologist, or equivalent professional, in conjunction with other clinical or laboratory findings.
- The reagents have not been tested on specimens from patients with minimal residual disease (MRD).

9. PERFORMANCE CHARACTERISTICS

Specimen handling and collection (AOB/AOS)

A study was performed to assess the Age of Blood (AOB) and Age of Stain (AOS) using BD OneFlow™ LST and BD OneFlow™ B-CLPD T1. The stability of EDTA- or heparin-anticoagulated peripheral blood, bone marrow, and lymph node specimens was evaluated by assessing the combined effect of:

- AOB: Time duration between specimen collection and staining
- AOS: Time duration between completion of stained sample processing and acquisition with the flow cytometer.

Specimens were tested up to 28 hours post collection and stained samples were tested up to 80 minutes post stain. All samples were maintained at room temperature before staining or acquisition.

Based on the results of this study, we recommend staining samples within 24 hours of collection and acquiring samples within 1 hour of staining if the sample is kept at 20–25 °C.

BD FACSLyric™ Flow Cytometer (BD OneFlow™ LST)

Method comparison, BD FACSLyric™ vs BD FACSCanto™ II flow cytometer (BD OneFlow™ LST)

A method comparison study between the BD OneFlow™ system on the BD FACSLyric™ flow cytometer (Investigational Method) and the BD OneFlow™ system on the BD FACSCanto™ II flow cytometer (Comparator Method) was performed at 4 clinical sites. The BD OneFlow™ system on BD FACSLyric™ comprises BD® CS&T Beads, BD® FC Beads 7-Color Kit, BD® FC Beads 5-Color Kit, and BD OneFlow™ LST. Stained samples were acquired on a 10-color BD FACSLyric™ flow cytometer (4-Blue 3-Red 3-Violet) using BD FACSuite™ Clinical application v1.3* and the BD OneFlow™ LST assay. The BD OneFlow™ reference system on BD FACSCanto™ II comprises BD FACSDiva™ CS&T IVD Beads, BD OneFlow™ Setup Beads, BD® FC Beads 8-Color Kit for BD OneFlow™ Assays, and BD OneFlow™ LST. Stained samples were acquired on a BD FACSCanto™ II flow cytometer (4-2H-2V) using BD FACSDiva™ software v8.0.2 and the BD OneFlow™ LST template. A total of 98 PB specimens, 26 BM specimens, and 16 LN specimens were enrolled in the study.

---

* A regression study was performed, demonstrating equivalence between BD FACSuite™ Clinical application v1.3 and v1.4.

Specimens were collected in the anticoagulants shown. See the following table.

Table 4 Anticoagulants used to collect specimens

Specimen type	Anticoagulant	
	EDTA	Heparin
PB	72	26
BM	23	3

For PB, BM, and LN specimens, the first wash step was started within 23, 24, or 23 hours of collection, respectively. Stained PB, BM, and LN samples were acquired within 50, 48, or 31 minutes of final resuspension, respectively. Samples were identified as being "Abnormal" (abnormal for B cells, T cells, or NK cells) or "Normal" (normal for T cells, B cells, and NK cells) using the two systems, and compared.

Agreement was calculated as follows:

Overall % agreement = ((a+d)/(a+b+c+d))×100
Positive % agreement = (a/(a+c))×100
Negative % agreement = (d/(d+b))×100

wherein,

a = number of samples "Abnormal" for both systems,
b = number of samples "Abnormal" on the BD FACSLyric™ flow cytometer but "Normal" on the BD FACSCanto™ II flow cytometer,
c = number of samples "Normal" on the BD FACSLyric™ flow cytometer but "Abnormal" on the BD FACSCanto™ II flow cytometer,
d = number of samples "Normal" for both systems.

The results for all cell types are shown in the following table.

Table 5 Agreement for all cells being "Abnormal" or "Normal"

		Comparator method (BD FACSCanto™ II flow cytometer)		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric™ flow cytometer)	Abnormal	63	0	63
	Normal	0	77	77
	Total	63	77	140

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for "Abnormal" (abnormal for B cells, T cells, and NK cells) is 100%. The negative agreement for "Normal" (normal for T cells, B cells, and NK cells) is 100%.

The results for B cells are shown in the following table.

Table 6 Agreement for B cells being "Abnormal" or "Normal"

		Comparator method (BD FACSCanto™ II flow cytometer)		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric™ flow cytometer)	Abnormal	52	0	52
	Normal	0	88	88
	Total	52	88	140

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for "Abnormal" (abnormal for B cells) is 100%. The negative agreement for "Normal" (normal for B cells) is 100%.

The results for T cells are shown in the following table.

Table 7 Agreement for T cells being "Abnormal" or "Normal"

		Comparator method (BD FACSCanto™ II flow cytometer)		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric™ flow cytometer)	Abnormal	9	0	9
	Normal	0	131	131
	Total	9	131	140

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for "Abnormal" (abnormal for T cells) is 100%. The negative agreement for "Normal" (normal for T cells) is 100%.

The results for NK cells are shown in the following table.

Table 8 Agreement for NK cells being "Abnormal" or "Normal" [a]

		Comparator method (BD FACSCanto™ II flow cytometer)		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric™ flow cytometer)	Abnormal	2	0	2
	Normal	0	138	138
	Total	2	138	140
a. NK cell malignancies are rare, therefore the expected number of samples is very low.				

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for "Abnormal" (abnormal for NK cells) is 100%. The negative agreement for "Normal" (normal for NK cells) is 100%.

Equivalency, BD FACSLyric™ vs BD FACSCanto™ II flow cytometer (BD OneFlow™ LST)

An assessment of the indicated cell populations, expressed as a percentage of another cell population, was performed for each evaluable specimen enrolled in the method comparison study. Specimens were analyzed using the BD OneFlow™ system on the BD FACSLyric™ flow cytometer and the BD FACSCanto™ II flow cytometer as described previously.

The mean bias for the percentages of the indicated cell populations determined on the BD FACSLyric™ flow cytometer versus the BD FACSCanto™ II flow cytometer was calculated. See the following table.

Table 9 Summary of mean bias for subset percentages

Population	No. of samples	Mean bias	Lower 95% CL of mean bias	Upper 95% CL of mean bias
Leukocytes (%SSC singlets)	134	−0.27	−0.73	0.19
Lymphocytes (%Leukocytes)	134	−0.63	−1.2	−0.06
T cells (%Lymphocytes)	134	−0.17	−0.58	0.24
CD4+CD8− cells (%T cells)	134	−0.53	−1.03	−0.04
CD8+CD4− cells (%T cells)	134	0.2	−0.41	0.81
B cells (%Lymphocytes)	134	−0.26	−0.72	0.19
sIgκ+ cells (%B cells)	134	−1.19	−2.1	−0.29
sIgλ+ cells (%B cells)	134	0.7	0.02	1.38
NK cells (%Lymphocytes)	134	0.45	−0.07	0.98

The results of the method comparison and equivalency studies indicate that the two systems are substantially equivalent.

Method comparison, BD FACS™ Universal Loader vs manual acquisition (BD OneFlow™ LST)

A study was performed at one site to demonstrate equivalency between acquisition using the BD FACS™ Universal Loader and manual acquisition. Peripheral blood or bone marrow specimens from normal and abnormal subjects were stained using 1 lot of BD OneFlow™ LST. For each specimen, 10 stained replicates were acquired either manually or using the BD FACS™ Universal Loader (using the 30-tube and 40-tube racks).

The mean, absolute bias, and relative bias for acquisition using the BD FACS™ Universal Loader vs manual acquisition were determined for the indicated populations. See the following table.

Table 10 BD FACS™ Universal Loader vs manual acquisition for BD OneFlow™ LST

Population	N	Mean		Absolute % bias (95% CI)	Relative % bias (95% CI)
		Loader	Manual		
Leukocytes (%SSC singlets)	17	95.46	94.83	0.64 (0.07, 1.21)	0.7 (0.1, 1.3)
Lymphocytes (%Leukocytes)	17	29.79	29.52	0.27 (–0.14, 0.68)	0.9 (–1.4, 3.2)
B cells (%Lymphocytes)	17	20.39	20.48	–0.09 (–0.40, 0.22)	–0.8 (–6.0, 4.6)
sIgκ$^+$ cells (%B cells)	16	61.27	62.89	–0.47 (–1.79, 0.86)	0.6 (–3.8, 5.3)
sIgλ$^+$ cells (%B cells)	16	38.4	36.63	0.60 (–0.60, 1.79)	1.5 (–1.5, 4.6)
T cells (%Lymphocytes)	17	68.08	68.2	–0.12 (–0.65, 0.42)	0.6 (–0.7, 1.8)
TCRγδ$^+$ cells (%Lymphocytes)	17	4.34	4.4	–0.06 (–0.28, 0.16)	0.9 (–6.4, 8.7)
CD4$^+$CD8$^-$ (%T cells)	17	45.99	45.95	0.05 (–0.46, 0.55)	0.2 (–1.1, 1.7)
CD8$^+$CD4$^-$ (%T cells)	17	44.61	44.31	0.30 (–0.21, 0.81)	0.9 (–0.5, 2.2)
NK cells (%Lymphocytes)	17	9.86	10.08	–0.22 (–0.73, 0.29)	–3.0 (–9.4, 3.9)

The results were also assessed qualitatively by a hematopathologist for concordance between samples acquired manually and samples acquired using the Loader. All samples stained with BD OneFlow™ LST showed 100% agreement.

Precision (within-site, control material) (BD OneFlow™ LST on BD FACSLyric™)

A 21-day single-site precision study was performed to assess the within-site precision (repeatability and reproducibility) of BD OneFlow™ LST using control material. Estimates of precision were determined across three BD FACSLyric™ flow cytometers and a minimum of three operators by acquiring BD Multi-Check™ Control, stained in duplicate by each operator using three lots of BD OneFlow™ LST. Two separate runs were performed by each operator on each of the 21 tested days.

Twelve cell populations were identified as being a percentage of the parent population. Repeatability (within-run variability) and reproducibility (variability between runs, between days, between operators, between lots, and between instruments) were estimated. A total of 756 samples were analyzed. The mean, standard deviation (SD), and coefficient of variation (%CV) is presented for each population. In addition, the one-sided 95% confidence limit (Upper SD or Upper %CV) for the within-site precision was calculated.

Table 11 Summary of within-site precision of subset percentages for BD OneFlow™ LST

Population (% of positive)	Mean	Repeatability		Reproducibility		Within-site precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
CD19$^+$	13.51	0.26	1.90	0.22	0.02	0.38	2.82
CD3$^+$	74.03	0.29	0.39	0.40	0.01	0.90	1.21
CD20$^+$	98.45	0.20	0.20	0.35	0.00	0.75	0.77
CD45$^+$	100.00	0.00	0.00	0.00	0.00	0.00	0.00
Igκ$^+$	62.42	0.82	1.32	0.89	0.01	1.69	2.70
Igλ$^+$	37.41	0.83	2.21	0.90	0.02	1.73	4.62
TCRγ/δ$^+$	2.94	0.11	3.70	0.11	0.04	0.20	6.67
CD56$^+$	98.42	0.23	0.23	0.17	0.00	0.34	0.35
CD4$^+$	69.95	0.31	0.44	0.16	0.00	0.41	0.59
CD5$^+$	74.33	0.28	0.38	0.61	0.01	1.64	2.21
CD8$^+$	27.10	0.30	1.11	0.49	0.02	1.37	5.07
CD38$^+$	47.53	0.64	1.34	5.34	0.11	6.26	13.17

Precision (reproducibility, control material) (BD OneFlow™ LST on BD FACSLyric™)

A study was performed at three sites to assess the reproducibility of the system. One lot of BD OneFlow™ LST was used to stain three replicates of BD Multi-Check™ Control. Two runs per day were acquired on a BD FACSLyric™ flow cytometer by one operator per site over a period of 5 days.

Twelve cell populations were identified as being a percentage of the parent population. Repeatability (within-run variability) and reproducibility (variability between runs, between days, and between sites) were estimated. The mean, standard deviation (SD), and coefficient of variation (%CV) is presented for each population. A total of 90 samples were analyzed. In addition, the one-sided 95% confidence limit (Upper SD or Upper %CV) for the overall precision was calculated.

Table 12 Summary of multi-site precision of subset percentages for BD OneFlow™ LST

Population (% of positive)	Mean	Repeatability		Reproducibility		Overall precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
CD19$^+$	13.85	0.24	1.76	0.07	0.00	0.29	2.08
CD3$^+$	73.57	0.31	0.42	0.48	0.01	1.32	1.80
CD20$^+$	99.12	0.14	0.14	0.13	0.00	0.24	0.25
CD45$^+$	35.59	0.37	1.04	0.64	0.02	1.27	3.56
Igκ$^+$	57.98	0.75	1.29	0.65	0.01	1.17	2.01
Igλ$^+$	41.65	0.83	1.99	0.85	0.02	1.41	3.38

Population (% of positive)	Mean	Repeatability		Reproducibility		Overall precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
TCRγ/δ⁺	96.10	0.16	0.17	0.19	0.00	0.39	0.40
CD56⁺	98.51	0.31	0.31	0.28	0.00	0.69	0.70
CD4⁺	66.21	0.28	0.42	0.21	0.00	0.41	0.62
CD5⁺	73.89	0.39	0.52	0.51	0.01	1.24	1.67
CD8⁺	30.76	0.34	1.09	0.31	0.01	0.75	2.45
CD38⁺	92.27	0.32	0.35	0.20	0.00	0.47	0.51

Detection capability (BD OneFlow™ LST)

The detection capability of BD OneFlow™ LST was assessed. An abnormal specimen was added to a hematologically normal peripheral blood specimen such that the abnormal population was at 0%, 0.02%, 0.05%, or 0.1% of all events of the normal specimen. Two sets of abnormal and normal specimens and 2 lots of BD OneFlow™ LST were evaluated in the study.

Table 13 Detection capability of BD OneFlow™ LST

Sample	Target population	Expected % abnormal cells	Mean actual % abnormal cell (95% CI)	SD of bias (Upper 95% CI)	Ratio[a]
1	CD45^{dim} CD19⁺ CD20^{dim} Igκ⁺ CD5⁺	0.0	0.003 (0.004, 0.002)	0.001 (0.003)	0/0
		0.02	0.026 (0.030, 0.022)	0.005 (0.01)	10/10
		0.05	0.061 (0.067, 0.056)	0.008 (0.014)	10/10
		0.1	0.118 (0.130, 0.105)	0.017 (0.032)	10/10
2	CD45^{dim} CD19⁺ CD20^{dim} Igκ⁺ CD5⁺	0.0	0.00 (0.001, 0.000)	0.000 (0.001)	0/0
		0.02	0.018 (0.022, 0.014)	0.006 (0.01)	10/10
		0.05	0.043 (0.048, 0.037)	0.008 (0.014)	10/10
		0.1	0.111 (0.122, 0.100)	0.015 (0.028)	10/10

a. Expected/actual number of positive replicates.

Based on the results, a detection capability of 0.03% of all events is recommended for BD OneFlow™ LST.

BD FACSLyric™ Flow Cytometer (BD OneFlow™ B-CLPD T1)

Method comparison, BD FACSLyric™ vs BD FACSCanto™ II flow cytometer (BD OneFlow™ B-CLPD T1)

A method comparison study between the BD OneFlow™ system on the BD FACSLyric™ flow cytometer (Investigational Method) and the BD OneFlow™ system on the BD FACSCanto™ II flow cytometer (Comparator Method) was performed at 4 external clinical sites. The BD OneFlow™ system on BD FACSLyric™ comprises BD® CS&T Beads, BD® FC Beads 7-Color Kit, BD® FC Beads 5-Color Kit, BD OneFlow™ LST, and BD OneFlow™ B-CLPD T1. Stained samples were acquired on a 10-color BD FACSLyric™ flow cytometer (4-Blue 3-Red 3-Violet) using BD FACSuite™ Clinical application v1.3† and the BD OneFlow™ B-CLPD T1 assay. The BD OneFlow™ reference system on BD FACSCanto™ II comprises BD FACSDiva™ CS&T IVD Beads, BD OneFlow™ Setup Beads, BD® FC Beads 8-Color Kit for BD OneFlow™ Assays, and BD OneFlow™ B-CLPD T1. Stained samples were acquired on a BD FACSCanto™ II flow cytometer (4-2H-2V) using BD FACSDiva™ software v8.0.2 and the BD OneFlow™ B-CLPD T1 template. A total of 93 evaluable PB specimens, 26 evaluable BM specimens, and 16 evaluable LN specimens were enrolled in the study.

† A regression study was performed, demonstrating equivalence between BD FACSuite™ Clinical application v1.3 and v1.4.

Specimens were collected in the anticoagulants shown. See the following table.

Table 14 Anticoagulants used to collect specimens

Specimen type	Anticoagulant	
	EDTA	Heparin
PB	68	25
BM	23	3

For PB, BM, and LN specimens, the first wash step was started within 23, 24, or 23 hours of collection, respectively. Stained PB, BM, and LN samples were acquired within 58, 49, or 34 minutes of final resuspension, respectively. Samples with aberrant B cell populations were identified as "CLL" or "Other B-CLPD" (including presumptive cases of atypical CLL) using the two systems. Samples were analyzed as "CLL" or "Non-

CLL" using the two systems, and compared. "Non-CLL" includes "Other B-CLPD", abnormal T, abnormal NK, and "normal" (normal for T cells, B cells, and NK cells). Furthermore, samples were analyzed as "Other B-CLPD" or Non-Other B-CLPD" using the two systems, and compared. "Non-Other B-CLPD" includes CLL, abnormal T, abnormal NK, and "normal" (normal for T cells, B cells, and NK cells).

Agreement was calculated as follows:

Overall % agreement = $((a+d)/(a+b+c+d)) \times 100$
Positive % agreement = $(a/(a+c)) \times 100$
Negative % agreement = $(d/(d+b)) \times 100$

wherein,

a = number of samples identified as "CLL" for both systems,
b = number of samples identified as "CLL" on the BD FACSLyric™ flow cytometer but identified as "Non-CLL" on the BD FACSCanto™ II flow cytometer,
c = number of samples identified as "Non-CLL" on the BD FACSLyric™ flow cytometer but identified as "CLL" on the BD FACSCanto™ II flow cytometer,
d = number of samples identified as "Non-CLL" for both systems.

The results for specimens identified as "CLL" or "Non-CLL" were tabulated. See the following table.

Table 15 Agreement for specimens identified as "CLL" or "Non-CLL"

		Comparator method (BD FACSCanto™ II flow cytometer)		
		CLL	Non-CLL	Total
Investigational method (BD FACSLyric™ flow cytometer)	CLL	25	0	25
	Non-CLL	0	110	110
	Total	25	110	135

Overall % agreement is 100%. The lower 95% confidence limit is 97.81%.

The positive agreement for "CLL" is 100%. The negative agreement for "Non-CLL" is 100%.

Agreement was also calculated using the formulas described previously wherein,

a = number of samples identified as "Other B-CLPD" for both systems,
b = number of samples identified as "Other B-CLPD" on the BD FACSLyric™ flow cytometer but identified as "Non-Other B-CLPD" on the BD FACSCanto™ II flow cytometer,
c = number of samples identified as "Non-Other B-CLPD" on the BD FACSLyric™ flow cytometer but identified as "Other B-CLPD" on the BD FACSCanto™ II flow cytometer,
d = number of samples identified as "Non-Other B-CLPD" for both systems.

The results for specimens identified as "Other B-CLPD" or "Non-Other B-CLPD" were tabulated. See the following table.

Table 16 Agreement for specimens identified as "Other B-CLPD" or "Non-Other B-CLPD"

		Comparator method (BD FACSCanto™ II flow cytometer)		
		Other B-CLPD	Non-Other B-CLPD	Total
Investigational method (BD FACSLyric™ flow cytometer)	Other B-CLPD	27	0	27
	Non-Other B-CLPD	0	108	108
	Total	27	108	135

Overall % agreement is 100%. The lower 95% confidence limit is 97.81%.

The positive agreement for "Other B-CLPD" is 100%. The negative agreement for "Non-Other B-CLPD" is 100%.

24

Equivalency, BD FACSLyric™ vs BD FACSCanto™ II flow cytometer (BD OneFlow™ B-CLPD T1)

A qualitative assessment of the relative fluorescence intensity, either positive or negative, of the indicated markers in aberrant B cells was performed for each evaluable specimen enrolled in the method comparison study. Specimens were analyzed using the BD OneFlow™ system on the BD FACSLyric™ flow cytometer and the BD FACSCanto™ II flow cytometer as described previously. Overall agreement of the two systems in assessing the expression, either positive or negative, of the indicated markers in the specimens was calculated. See the following table.

Table 17 Agreement for qualitative marker expression on BD FACSLyric™ and BD FACSCanto™ II flow cytometers

Marker	BD OneFlow™ reagent tube	% Agreement for the expression of marker	Lower 95% CL of % agreement
CD5	LST	98.08	91.20
CD20	LST	100.0	94.4
CD20	B-CLPD T1	100.0	94.4
CD200	B-CLPD T1	100.0	94.4
CD23	B-CLPD T1	100.0	94.4
CD79b	B-CLPD T1	100.0	94.4
CD10	B-CLPD T1	100.0	94.4
CD43	B-CLPD T1	96.15	88.38

The results of the method comparison and equivalency studies indicate that the two systems are substantially equivalent.

Method comparison, BD FACS™ Universal Loader vs manual acquisition (BD OneFlow™ B-CLPD T1)

A study was performed at one site to demonstrate equivalency between acquisition using the BD FACS™ Universal Loader and manual acquisition. Peripheral blood or bone marrow specimens from normal and abnormal subjects were stained using 1 lot of BD OneFlow™ B-CLPD T1. For each specimen, 10 stained replicates were acquired either manually or using the BD FACS™ Universal Loader (using the 30-tube and 40-tube racks).

The mean, absolute bias, and relative bias for acquisition using the BD FACS™ Universal Loader vs manual acquisition were determined for the indicated populations. See the following table.

Table 18 BD FACS™ Universal Loader vs manual acquisition for BD OneFlow™ B-CLPD T1

Population	N	Mean		Absolute % bias (95% CI)	Relative % bias (95% CI)
		Loader	Manual		
Leukocytes (%SSC singlets)	17	93.56	92.89	0.67 (0.12, 1.22)	0.8 (0.2, 1.4)
Lymphocytes (%Leukocytes)	17	27.84	27.84	0.00 (–0.71, 0.71)	0.2 (–2.3, 2.7)
B cells (%Lymphocytes)	17	19.97	20.04	–0.06 (–0.46, 0.33)	0.4 (–2.6, 3.6)

The results were also assessed qualitatively by a hematopathologist for concordance between samples acquired manually and samples acquired using the Loader. All samples stained with BD OneFlow™ B-CLPD T1 showed 100% agreement.

Precision (within-site, control material) (BD OneFlow™ B-CLPD T1 on BD FACSLyric™)

A 21-day single-site precision study was performed to assess the within-site precision (repeatability and reproducibility) of BD OneFlow™ B-CLPD T1 using control material. Estimates of precision were determined across three BD FACSLyric™ flow cytometers and a minimum of three operators by acquiring CD-Chex Plus® control, stained in duplicate by each operator using three lots of BD OneFlow™ B-CLPD T1. Two separate runs were performed by each operator on each of the 21 tested days.

Eight cell populations were identified as being a percentage of the parent population. Repeatability (within-run variability) and reproducibility (variability between runs, between days, between operators, between lots, and between instruments) were estimated. A total of 756 samples were analyzed. The mean, standard deviation (SD), and coefficient of variation (%CV) is presented for each population. In addition, the one-sided 95% confidence limit (Upper SD or Upper %CV) for the within-site precision was calculated.

Table 19 Summary of with-site precision of subset percentages for BD OneFlow™ B-CLPD T1

Population (% of positive)	Mean	Repeatability		Reproducibility		Within-site precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
CD43+	85.73	0.20	0.24	0.23	0.00	0.43	0.51
CD45+	42.16	1.15	2.72	0.53	0.01	1.34	3.19
CD10+	97.87	0.22	0.23	0.34	0.00	0.72	0.73
CD79b+	13.45	0.19	1.44	0.46	0.03	1.31	9.77
CD19+	13.91	0.19	1.39	0.08	0.01	0.23	1.63
CD20+	13.68	0.19	1.41	0.27	0.02	0.62	4.55
CD200+	11.00	0.17	1.56	0.25	0.02	0.54	4.92
CD23+	12.91	0.19	1.51	0.61	0.05	1.92	14.85

Precision (reproducibility, control material) (BD OneFlow™ B-CLPD T1 on BD FACSLyric™)

A study was performed at three sites to assess the reproducibility of the system. One lot of BD OneFlow™ BCLPD T1 was used to stain three replicates of CD-Chex Plus® control. Two runs per day were acquired on a BD FACSLyric™ flow cytometer by one operator per site over a period of 5 days.

Eight cell populations were identified as being a percentage of the parent population. Repeatability (within-run variability) and reproducibility (variability between runs, between days, and between sites) were estimated. A total of 90 samples were analyzed.The mean, standard deviation (SD), and coefficient of variation (%CV) is presented for each population. In addition, the one-sided 95% confidence limit (Upper SD or Upper %CV) for the overall precision was calculated.

Table 20 Summary of multi-site precision of subset percentages for BD OneFlow™ B-CLPD T1

Population (% of positive)	Mean	Repeatability		Reproducibility		Overall precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
CD43+	86.20	0.20	0.23	0.29	0.00	0.80	0.92
CD45+	39.06	0.43	1.10	0.73	0.02	1.11	2.84
CD10+	97.89	0.71	0.73	0.63	0.01	1.50	1.53
CD79b+	13.10	0.21	1.63	0.21	0.02	0.49	3.75
CD19+	13.26	0.21	1.56	0.16	0.01	0.39	2.96
CD20+	13.15	0.21	1.59	0.19	0.01	0.46	3.52
CD200+	10.24	0.18	1.75	0.28	0.03	0.75	7.36
CD23+	12.92	0.21	1.65	0.13	0.01	0.32	2.50

Detection capability (BD OneFlow™ B-CLPD T1)

The detection capability of BD OneFlow™ B-CLPD T1 was assessed. An abnormal specimen was added to a hematologically normal peripheral blood specimen such that the abnormal population was at 0%, 0.02%, 0.05%, or 0.1% of all events of the normal specimen. Two sets of abnormal and normal specimens and 2 lots of BD OneFlow™ BCLPD T1 were evaluated in the study.

Table 21 Detection capability of BD OneFlow™ B-CLPD T1

Sample	Target population	Expected % abnormal cells	Mean actual % abnormal cell (95% CI)	SD of bias (Upper 95% CI)	Ratio[a]
1	$CD45^{dim}$ $CD19^{+}$ $CD20^{dim}$ CD43+ CD200+	0.0	0 (0.000, 0.000)	0 (0.001)	0/0
		0.02	0.027 (0.031, 0.022)	0.006 (0.011)	10/10
		0.05	0.054 (0.061, 0.046)	0.010 (0.019)	10/10
		0.1	0.111 (0.123, 0.099)	0.016 (0.030)	10/10
2	$CD45^{dim}$ $CD19^{+}$ $CD20^{dim}$ CD43+ CD200+	0.0	0 (0.001, 0.000)	0 (0.001)	0/0
		0.02	0.02 (0.023, 0.018)	0.003 (0.006)	10/10
		0.05	0.053 (0.058, 0.048)	0.007 (0.012)	10/10
		0.1	0.102 (0.110, 0.093)	0.012 (0.022)	10/10

a. Expected/actual number of positive replicates.

27

Based on the results, a detection capability of 0.03% of all events is recommended for BD OneFlow™ B-CLPD T1.

BD FACSCanto™ II Flow Cytometer (BD OneFlow™ LST)

Method comparison, BD FACSCanto™ II flow cytometer vs EuroFlow system (BD OneFlow™LST)

A side-by-side comparison study between the BD OneFlow™ LST system on the BD FACSCanto™ II flow cytometer and the EuroFlow LST system on the BD FACSCanto™ II flow cytometer was performed at 3 external clinical sites. The BD OneFlow™ LST system comprises BD OneFlow™ Setup Beads, BD® FC Beads for compensation, and the BD OneFlow™ LST reagent. The EuroFlow LST reference system comprises Sphero™ Rainbow calibration particles (8 peaks), single color stained cells plus BD® Multicolor CompBeads for compensation, and the EuroFlow LST reagent cocktail. Both methods used BD FACSDiva™ CS&T IVD beads to perform instrument quality control. Abnormal mature lymphocyte populations from 81 patients with B-cell malignancies, 35 patients with T-cell malignancies, and 6 patients with NK-cell malignancies were identified using the two systems, and compared. In addition, 9 samples were identified as being from other lineages, for example, plasma cell disorders or bi-phenotypic samples. Cell populations from 76 negative samples, including 19 healthy donors, were identified using the two systems. A total of 123 PB specimens, 53 BM specimens, and 31 fresh LN specimens were enrolled in the study. PB and BM specimens were stained within 24 hours of collection. LN specimens were stained within 6 hours of collection. All stained samples were acquired within 1 hour of staining. Samples were identified as being "Follow-up needed" or "No follow-up needed" using the two systems, and compared.

Agreement was calculated as follows:

Overall % agreement = ((a+d)/(a+b+c+d))×100

wherein,

a = number of samples "Follow-up needed" for both systems,
b = number of samples "Follow-up needed" for the BD OneFlow™ system but "No follow-up needed" for the EuroFlow system,
c = number of samples "No follow-up needed" for the BD OneFlow™ system but "Follow-up needed" for the Euroflow system, and
d = number of samples "No follow-up needed" for both systems.

The results for all cell types are shown in the following table.

Table 22 Agreement for all cells being "Follow-up needed" or "No follow-up needed"

		Comparator method (Euroflow LST cocktail)		
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow™ LST)	Follow-up needed	131	0	131
	No follow-up needed	0	76	76
	Total	131	76	207

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

The results for T cells are shown in the following table.

Table 23 Agreement for T cells being "Follow-up needed" or "No follow-up needed"

		Comparator method (Euroflow LST cocktail)		
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow™ LST)	Follow-up needed	35	0	35
	No follow-up needed	0	172	172
	Total	35	172	207

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

The results for B cells are shown in the following table.

Table 24 Agreement for B cells being "Follow-up needed" or "No follow-up needed"

		Comparator method (Euroflow LST cocktail)		
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow™ LST)	Follow-up needed	81	0	81
	No follow-up needed	0	126	126
	Total	81	126	207

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

The results for NK cells are shown in the following table.

Table 25 Agreement for NK cells being "Follow-up needed" or "No follow-up needed"[a]

		Comparator method (Euroflow LST cocktail)		
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow™ LST)	Follow-up needed	6	0	6
	No follow-up needed	0	201	201

		Comparator method (Euroflow LST cocktail)		
	Total	6	201	207

a. NK cell malignancies are rare, therefore the expected number of samples is very low.

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

Equivalency, BD FACSCanto™ II flow cytometer vs EuroFlow system (BD OneFlow™ LST)

Peripheral blood, bone marrow, and lymph node specimens collected at 3 external clinical laboratories were obtained from patients with T-cell, B-cell, or NK-cell abnormalities, or with no hematological abnormalities. Specimens were analyzed using the BD OneFlow™ LST system and the EuroFlow LST system described previously.

The bias for leukocytes identified as being a percentage of SSC singlets is summarized in the following table.

Table 26 Summary of bias for Leukocytes (%SSC singlets)

Population	No. of samples	Average bias	Lower 95% CL of average bias	Upper 95% CL of average bias
Leukocytes (%SSC singlets)	207	−1.1%	−1.7%	−0.6%

The remaining eight cell populations were identified as being a percentage of the cell populations indicated in the following table. Deming regression statistics indicate that the results obtained using the two systems are substantially equivalent.

Table 27 Equivalency of the BD OneFlow™ system to the EuroFlow system

Population	No. of samples	Intercept	Slope	Lower 95% CL of average bias	Upper 95% CL of average bias
Lymphocytes (%Leukocytes)	207	−0.68	1.01	1.00	1.02
T cells (%Lymphocytes)	207	1.07	0.99	0.98	1.00
$CD4^+CD8^-$ cells (%T cells)	207	−0.64	1.01	1.00	1.02
$CD8^+CD4^-$ cells (%T cells)	207	−0.45	1.00	0.99	1.01
B cells (%Lymphocytes)	207	−0.01	1.00	0.99	1.00
NK cells (%Lymphocytes)	207	0.81	0.92	0.84	1.00
smIgκ$^+$ cells (%B cells)	206[a]	2.24	1.00	0.97	1.02

Population	No. of samples	Intercept	Slope	Lower 95% CL of average bias	Upper 95% CL of average bias
smIgλ⁺ cells (%B cells)	206[a]	−2.04	1.00	0.98	1.03

a. One patient specimen had 0 and 2 B-cell events for the two systems, respectively. Since smIgκ and smIgλ are defined as a percentage of B-cell events in this study, they could not be defined in one system, and therefore could not be included in the analysis for smIgκ and smIgλ for that specimen.

Precision (reproducibility) (BD OneFlow™ LST on BD FACSCanto™ II)

Two operators performed two separate runs per day over a period of 8 days, alternating the runs on two BD FACSCanto™ II flow cytometers. For each run, duplicate samples of BD Multi-Check™ Control were stained using three lots of BD OneFlow™ LST by each operator, and then acquired and analyzed using the BD OneFlow™ LST template in BD FACSDiva™ software. Nine cell populations were identified as being a percentage of the cell populations indicated in the following table. The reproducibility of the subset percentages was calculated for each cell population. Reproducibility comprises four components: operator/instrument-to-operator/instrument, lot-to-lot, run-to-run, and day-to-day reproducibility.

Table 28 Reproducibility of subset percentages

Population	Mean	SD[a]	Upper 95% CL[b] of SD	%CV[c]	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	100.0	0.01	0.03	0.01	0.03
Lymphocytes (%Leukocytes)	37.9	0.5	1.1	1.2	2.9
T cells (%Lymphocytes)	70.3	0.2	0.3	0.3	0.5
CD4⁺CD8⁻ cells (%T cells)	63.4	0.5	1.5	0.8	2.4
CD8⁺CD4⁻ cells (%T cells)	25.1	1.1	3.2	4.4	12.8
B cells (%Lymphocytes)	14.8	0.2	1.0	1.5	6.5
NK cells (%Lymphocytes)	14.8	0.3	0.8	1.9	5.6
smIgκ⁺ cells (%B cells)	59.4	0.2	3.1	0.3	5.2
smIgλ⁺ cells (%B cells)	40.6	0.2	2.8	0.4	6.8

a. SD = Standard deviation
b. CL = Confidence limit
c. %CV = Coefficient of variation

Precision (repeatability) (BD OneFlow™ LST on BD FACSCanto™ II)

Two operators performed two separate runs per day over a period of 8 days, alternating the runs on two BD FACSCanto™ II flow cytometers. For each run, duplicate samples of BD Multi-Check™ Control were stained using three lots of BD OneFlow™ LST by each operator, and then acquired and analyzed using the BD OneFlow™ LST template in BD FACSDiva™ software. Nine cell populations were identified as being a percentage of the cell populations indicated in the following table. The within-run precision (tube-to-tube repeatability) of the subset percentages was calculated for each of the cell populations.

Table 29 Repeatability of subset percentages

Population	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	100.0	0.03	0.03	0.03	0.03
Lymphocytes (%Leukocytes)	37.9	0.5	0.5	1.3	1.4
T cells (%Lymphocytes)	70.3	0.4	0.4	0.5	0.6
CD4$^+$CD8$^-$ cells (%T cells)	63.4	0.5	0.6	0.8	0.9
CD8$^+$CD4$^-$ cells (%T cells)	25.1	0.7	0.8	3.0	3.2
B cells (%Lymphocytes)	14.8	0.3	0.3	1.8	2.0
NK cells (%Lymphocytes)	14.8	0.3	0.3	2.1	2.3
smIgκ$^+$ cells (%B cells)	59.4	0.8	0.8	1.3	1.4
smIgλ$^+$ cells (%B cells)	40.6	0.8	0.8	1.9	2.1

BD FACSCanto™ II Flow Cytometer (BD OneFlow™ B-CLPD T1)

Method comparison, BD FACSCanto™ II flow cytometer vs EuroFlow system (BD OneFlow™ B-CLPD T1)

A side-by-side comparison study between the BD OneFlow™ system on the BD FACSCanto™ II flow cytometer and the EuroFlow system on the BD FACSCanto™ II flow cytometer was performed at 4 external clinical sites. The BD OneFlow™ system comprises BD OneFlow™ Setup Beads, BD$^®$ FC Beads for compensation, the BD OneFlow™ LST reagent, and the BD OneFlow™ B-CLPD T1 reagent. The EuroFlow reference system comprises Sphero™ Rainbow calibration particles (8 peaks), single color stained cells plus BD$^®$ CompBead particles for compensation, and the corresponding EuroFlow reagent cocktails. Both methods used BD FACSDiva™ CS&T IVD beads to perform instrument quality control. Abnormal mature B-cell populations from 54 patients with CLL and 47 patients with other B-cell disorders, including presumptive cases of atypical CLL, were identified using the two systems, and compared. A total of 70 PB specimens and 31 BM specimens were enrolled in the study. PB and BM specimens were stained within 26 hours of collection. All stained samples were acquired within 66 minutes of staining. Samples with B cells needing follow-up were classified as CLL or another B-cell disorder.

Agreement was calculated as follows:

Overall % agreement = ((a+d)/(a+b+c+d))×100

wherein,

a = number of samples classified as CLL for both systems,
b = number of samples classified as CLL for the BD OneFlow™ system but classified as other B-cell disorder for the EuroFlow system,
c = number of samples classified as other B-cell disorder for the BD OneFlow™ system but classified as CLL for the Euroflow system, and
d = number of samples classified as other B-cell disorder for both systems.

The results for samples classified as CLL or another B-cell disorder were tabulated. See the following table.

Table 30 Agreement for B cells needing follow-up as CLL or other B-cell disorder

		Comparator method (Euroflow LST cocktail)		
		CLL	Other B-cell disorder	Total
Investigational method (BD OneFlow™ system)	CLL	54	0	54
	Other B-cell disorder	0	47	47
	Total	54	47	101

Overall % agreement is 100%. The lower 95% confidence limit is 97.1%.

Equivalency, BD FACSCanto™ II flow cytometer vs EuroFlow system (BD OneFlow™ B-CLPD T1)

Specimens with CD45$^+$CD19$^+$ B cells identified as needing follow-up using the BD OneFlow™ LST tube were further characterized according to the expression of specific markers. Specimens were analyzed using the BD OneFlow™ system and the corresponding EuroFlow system described previously. Agreement of the two systems in assessing the expression, either positive or negative, of the indicated markers in aberrant B cells was calculated. See the following table.

Table 31 Equivalency of the BD OneFlow™ system to the EuroFlow system

Marker	BD OneFlow™ reagent tube	% Agreement for the expression of marker	Lower 95% CL of % agreement
CD5	LST	98.0%	93.9%
CD20	LST	100.0%	97.1%
CD20	B-CLPD T1	100.0%	97.1%
CD200	B-CLPD T1	100.0%	97.1%
CD23	B-CLPD T1	100.0%	97.1%
CD79b	B-CLPD T1	99.0%	95.4%

The results of the method comparison and equivalency studies indicate that the two systems are substantially equivalent.

Precision (reproducibility) (BD OneFlow™ B-CLPD T1 on BD FACSCanto™ II)

Two operators performed two separate runs per day over a period of 8 days, alternating the runs on two BD FACSCanto™ II flow cytometers. For each run, duplicate samples of CD-Chex Plus® BC were stained using three lots of BD OneFlow™ B-CLPD T1 by each operator, and then acquired and analyzed using the BD OneFlow™ B-CLPD T1 template in BD FACSDiva™ software. Three cell populations were identified as being a percentage of the cell populations indicated in Table 32. The reproducibility of the subset percentages was

calculated for each cell population. Reproducibility comprises four components: operator/instrument-to-operator/instrument, lot-to-lot, run-to-run, and day-to-day reproducibility.

Table 32 Reproducibility of subset percentages

Population	Mean	SD[a]	Upper 95% CL[b] of SD	%CV[c]	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	100.00	0.00	0.00	0.00	0.00
Lymphocytes (%Leukocytes)	36.94	1.23	1.40	3.34	3.74
B cells (%Lymphocytes)	12.45	0.19	0.22	1.53	1.71

a. SD = Standard deviation
b. CL = Confidence limit
c. %CV = % Coefficient of variation

Precision (repeatability) (BD OneFlow™ B-CLPD T1 on BD FACSCanto™ II)

Two operators performed two separate runs per day over a period of 8 days, alternating the runs on two BD FACSCanto™ II flow cytometers. For each run, duplicate samples of CD-Chex Plus BC were stained using three lots of BD OneFlow™ B-CLPD T1 by each operator, and then acquired and analyzed using the BD OneFlow™ B-CLPD T1 template in BD FACSDiva™ software. Three cell populations were identified as being a percentage of the cell populations indicated in the following table. The within-run precision (tube-to-tube repeatability) of the subset percentages was calculated for each cell population.

Table 33 Repeatability of subset percentages

Population	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	100.00	0.00	0.00	0.00	0.00
Lymphocytes (%Leukocytes)	36.94	0.40	0.45	1.08	1.21
B cells (%Lymphocytes)	12.45	0.19	0.22	1.52	1.70

10. TROUBLESHOOTING

Problems with cell preparation or staining

Problem	Possible cause	Solution
The resolution between debris and lymphocytes is poor.	Specimen was poorly lysed.	Prepare and stain another specimen.
	Specimen is of poor quality.	Check cell viability.
	Specimen is too old.	Obtain a new specimen and stain it immediately.
Staining is dim or fading.	Cell concentration was too high at the staining step.	Check the cell concentration and adjust as needed.
	Washed specimen was not stained within 30 minutes of the last wash.	Repeat staining with a freshly prepared specimen.

Problem	Possible cause	Solution
	The reagent tube was exposed to light for too long.	Repeat staining with a new tube of reagent.
	Stained cells were stored too long before acquiring them.	Repeat staining with a fresh specimen and acquire it promptly.
Few or no cells are recorded.	Cell concentration was too low.	Centrifuge specimen and resuspend it at a higher concentration. Repeat staining and acquisition.
	Cytometer is malfunctioning.	Troubleshoot the instrument. See the cytometer IFU for more information.

Problems using the reagent on BD FACSLyric™ flow cytometers

Problem	Possible cause	Solution
Not enough cells of interest are acquired.	Cell concentration was too low.	Centrifuge specimen and resuspend it at a higher concentration. Repeat staining and acquisition.
	The default setting of 100,000 events acquired is too low.	Change the number of events acquired. Repeat staining and acquisition. See the *BD OneFlow™ Application Guide for Chronic Lymphoproliferative Diseases for BD FACSLyric™ Flow Cytometer*s.
The FSC-A vs SSC-A dot plot is abnormal.	Cytometer needs adjusting.	Contact BD Biosciences.
The csv file and report are not exported automatically.	The reagent lot number and expiration date were not added to the Library.	1. Add the reagent lot number and expiration date to the Library. 2. Export the csv file and the report PDF manually. See the *BD FACSLyric™ System Instructions For Use*.

Problems using the reagents on BD FACSCanto™ II flow cytometers

Problem	Possible cause	Solution
The resolution between debris and lymphocytes is poor.	Instrument settings are inappropriate.	Follow proper instrument setup procedures. See the *Instrument Setup Guide for BD OneFlow™ Assays*.
Some of the dot plots are dimmed.	FSC-H and SSC-H were not selected when the application settings were created.	Check that FSC-H and SSC-H are selected on the Parameters tab of theInspector.
The barcode on the BD OneFlow™ tube label cannot be scanned.	The barcode on the tube label has been compromised.	Scan the barcode on the

Problem	Possible cause	Solution
		BD OneFlow™ pouch label into the Product ID keyword field in the Experiment Layout. Next, manually enter a semicolon (;) followed by the six-digit tube-specific ID, found adjacent to the barcode on the tube label, after the last digit of the barcode.
The dot plots on the worksheets are missing or the dot plots do not have gates.	The template did not import correctly.	1. Close the current experiment. 2. Create a new experiment. 3. Re-import the BD OneFlow™ LST template.
Some of the keywords are missing from the statistics box in the analysis worksheet.	BD FACSDiva™ software did not import all of the keywords into the panel template.	1. In the Browser, set the current tube pointer to the tube that you are analyzing. 2. Navigate to the analysis worksheet. 3. Right-click the statistics box and select Edit Stats View. 4. In the Header tab, select the All checkbox. 5. Click **OK**.
The statement, For in vitro diagnostic use, does not appear in the footer of the analysis worksheet when it is printed.	The paper margins in the printer settings were changed.	1. From the BD FACSDiva™ software menu bar, select **File > Page Setup**. 2. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. 3. Click **OK**.

REFERENCES

1. van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia.* 2012;26:1908-1975.
2. Bernard A, Boumsell L, Hill C. Joint report of the first international workshop on human leucocyte differentiation antigens by the investigators of the participating laboratories. In: Bernard A, Boumsell L, Dausset J, Milstein C, Schlossman SF, eds. *Leucocyte Typing.* New York, NY: Springer-Verlag; 1984:9-108.
3. Lanier LL, Le AM, Civin CI, Loken MR, Phillips JH. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J Immunol.* 1986;136:4480-4486.
4. Ritz J, Trinchieri G, Lanier LL. NK-cell antigens: section report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens.* Vol 2. New York, NY: Oxford University Press; 1995:1367-1372.

5. Engleman EG, Warnke R, Fox RI, Dilley J, Benike CJ, Levy R. Studies of a human T lymphocyte antigen recognized by a monoclonal antibody. *Proc Natl Acad Sci USA*. 1981;78:1791-1795.

6. Knowles RW. Immunochemical analysis of the T-cell-specific antigens. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human T Lymphocytes.* Vol 1. New York, NY: Springer-Verlag; 1986:259-288.

7. Ling NR, Maclennan ICM, Mason DY. B-cell and plasma cell antigens: new and previously defined clusters. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:302-335.

8. Nadler LM. B Cell/Leukemia Panel Workshop: Summary and Comments. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human B Lymphocytes.* Vol 2. New York, NY: Springer-Verlag; 1986;3-43.

9. Cobbold SP, Hale G, Waldmann H. Non-lineage, LFA-1 family, and leucocyte common antigens: new and previously defined clusters. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:788-803.

10. Appendix C. Summary of antibody names, code numbers, and donor laboratories. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1987:988-993.

11. Kubagawa H, Gathings WE, Levitt D, Kearney JF, Cooper MD. Immunoglobulin isotype expression of normal pre-B cells as determined by immunofluorescence. *J Clin Immunol*. 1982;2(4):264-269.

12. Borst J, van Dongen J, Bolhuis R, et al. Distinct molecular forms of human T cell receptor γ/δ detected on viable T cells by a monoclonal antibody. *J Exp Med*. 1988;167:1625-1644.

13. Ichinohasama R, Miura I, Takahashi T, et al. Peripheral CD4+ CD8– γδ T cell lymphoma: a case report with multiparameter analyses. *Hum Pathol*. 1996;27:1370-1377.

14. Sarfati M, Ishihara H, Delespesse G. CD23 Workshop Panel report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1995:530-533.

15. Zola H. CD10 Workshop Panel report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1995:505-507.

16. Engel P, Wagner N, Tedder TF. CD79 Workshop report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1995:667-679.

17. Cerný J, Angelisová P, Horejší V. Non-lineage Panel—Analysis by Western blotting. In: Mason D, André P, Bensussan A, et al, eds. *Leucocyte Typing VII: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 2002:458.

18. Hilgert I, Drbal K. Non-lineage Panel—Analysis by cytofluorometry. In: Mason D, André P, Bensussan A, et al, eds. *Leucocyte Typing VII: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 2002:459-461.

19. Horejší V, Stockinger H. CD43 Workshop Panel report. In: Kishimoto H, Kikutani H, von dem Borne AEGK, et al, eds. *Leucocyte Typing VI: White Cell Differentiation Antigens. New York: Garland Publishing, Inc.*; 1998:494-497.

20. *Collection of Diagnostic Venous Blood Specimens*, *7th ed.* Wayne, PA: Clinical and Laboratory Standards Institute; 2017. CLSI document GP41.

21. Rothe G, Schmitz G. Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies. *Leukemia*. 1996;10:877-895.

22. Stelzer GT, Marti G, Hurley A, McCoy PJ, Lovett EJ, Schwartz A. US-Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: standardization and validation of laboratory procedures. *Cytometry*. 1997;30:214-230.

23. *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2007. CLSI document H43-A2.

24. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.
25. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.

NOTICE

EU Only: Users shall report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

Refer to the Eudamed website: https://ec.europa.eu/tools/eudamed for Summary of Safety and Performance.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD'S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

TRADEMARKS

BD, the BD Logo, BD FACSDiva, BD FACSLyric, BD FACSuite, BD Multi-Check, BD OneFlow, FACS, FACSCanto, Horizon and Vacutainer are trademarks of Becton, Dickinson and Company or its affiliates. All other trademarks are the property of their respective owners. © 2022 BD. All rights reserved.

Cy™ is a trademark of GE Healthcare. This product is subject to proprietary rights of GE Healthcare and Carnegie Mellon University, and is made and sold under license from GE Healthcare. This product is licensed for sale only for in vitro diagnostics. It is not licensed for any other use. If you require any additional license to use this product and do not have one, return this material, unopened, to BD Biosciences, 2350 Qume Drive, San Jose, CA 95131, and any money paid for the material will be refunded.

The EuroFlow trademark and logo and the EuroFlow™ antibody panels are property of the EuroFlow Consortium and cannot be reproduced or published without prior written permission from the EuroFlow coordinator (euroflow.org).

HISTORY

Revision	Date	Changes made
23-15586-02	2020-05	Revised to support using the product on BD FACSLyric™ flow cytometers.
23-15586-03	2021-01	Revised to support using the product with the BD FACS™ Universal Loader.
23-15586(06)	2022-06	Updated to meet requirements of Regulation (EU) 2017/746. Merged BD OneFlow™ LST and BD OneFlow™ B-CLPD T1 IFUs to support the panel.

SYMBOLS GLOSSARY [L006715(06) 2021-08]

Some symbols listed below may not apply to this product.
US Customers only: For symbol glossary, refer to **bd.com/symbols-glossary**

Symbol	Meaning
	Manufacturer
EC REP	Authorized representative in the European Community
CH REP	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
LOT	Batch code
REF	Catalogue number
SN	Serial number
STERILE	Sterile
STERILE A	Sterilized using aseptic processing techniques
STERILE EO	Sterilized using ethylene oxide
STERILE R	Sterilized using irradiation
STERILE	Sterilized using steam or dry heat
	Do not resterilize
	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
STERILE	Sterile fluid path
STERILE EO	Sterile fluid path (ethylene oxide)
STERILE R	Sterile fluid path (irradiation)
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Lower limit of temperature
	Upper limit of temperature
	Temperature limit
	Humidity limitation
	Biological risks
	Do not re-use
	Consult *instructions for use* or consult electronic *instructions for use*
	Caution
LATEX	Contains or presence of natural rubber latex
IVD	In vitro diagnostic medical device
CONTROL −	Negative control
CONTROL +	Positive control
	Contains sufficient for *<n>* tests
	For IVD performance evaluation only
	Non-pyrogenic

Symbol	Meaning
#	Patient number
	This way up
	Do not stack
	Single sterile barrier system
PHT DEHP BBP	Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP)
	Collect separately Indicates separate collection for waste of electrical and electronic equipment required.
CE	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
R_x Only	This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner."
CC	Country of manufacture "CC" shall be replaced by either the two letter or the three letter country code.
	Collection time
	Cut
	Peel here
	Collection date
	Keep away from light
H_2	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Internal sequence number
MD	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
FC	Meets FCC requirements per 21 CFR Part 15
c(UL)us	UL product certification for US and Canada
UDI	Unique device identifier

39

CONTACT INFORMATION

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, California 95131 USA

EC REP **Becton Dickinson Ireland Ltd.**
Donore Road, Drogheda
Co. Louth, A92 YW26
Ireland

CH REP **BD Switzerland Sàrl**
Terre Bonne Park – A4
Route de Crassier 17
1262 Eysins, Switzerland

BD Biosciences
European Customer Support
Tel +32.53.720.600
help.biosciences@bd.com

Australian and New Zealand Distributors:

Becton Dickinson Pty Ltd.
66 Waterloo Road
Macquarie Park NSW 2113
Australia

Becton Dickinson Limited
14B George Bourke Drive
Mt. Wellington Auckland 1060
New Zealand

Technical Service and Support: Contact
your local BD representative or
bdbiosciences.com.

ClinicalApplications@bd.com

eifu.bd.com